

## REMARKS

### **I. Detailed Action**

Claims 14-16, 18 and 20 are under consideration in this examination.

The Examiner states the oath or declaration is defective. New oath or declaration in compliance with 37 C.F.R. § 1.67(a) identifying this application by Application No. and filing date is required.

Applicants are submitting separately a new oath or declaration thereby making this objection moot.

### **II. Drawings**

The Draftsman's objections to the drawings are enclosed in the notice on form PTO—948. Correction is required.

Applicants are submitting herein corrected drawings which include black and white photographs. Pursuant to 37 CFR 1.84, photographs are submitted because the invention is shown more clearly in them.

### **III. Abstract**

The Examiner has objected to the instant abstract because it exceeds 25 lines, even though there are less than 250 words.

Applicants have amended the abstract such that it will not exceed 25 lines.

The Examiner states that the specification has not been checked to the extent necessary to determine the presence of all possible minor errors. Applicants' cooperation is requested in correcting any errors of which Applicants may become aware in the specification.

Applicants have attempted to correct minor errors that they have become aware of in the specification.

The Examiner states the Amendment filed June 29, 2001 (Paper No. 6) is objected to under 35 U.S.C. § 132 because it introduces new matter into the disclosure. The added material which is not supported by the original disclosure is as follows:

No method for the identification of nucleic acid comprising the method steps of claim 14-16, 18 and 20 have been described in the specification or have any basis in the specification, as originally filed. Similarly, no oligonucleotide fragments of contiguous bases of SEQ ID NO: 1 or "computer algorithm" has been previously described in the specification or has any basis in the specification, as originally filed. Applicant is required to cancel this new matter in the reply to this Office Action.

Applicants have cancelled the new matter in this reply to the Office Action, making this objection moot.

#### **IV. Claim Rejections--35 U.S.C. § 112**

Claims 14-16, 18 and 20 were rejected under 35 U.S.C. § 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s) at the time the application was filed had possession of the claimed invention.

The Examiner states there are no specific oligo-fragments or hybridization conditions or what amino acid substitution is considered as conservative modification (variants) are described. No representative number of species corresponding to nucleotide of SEQ ID NO: 1 encoding expansin from other species and/or structure activity relationships are disclosed. There is no prior art disclosure of polynucleotide sequences encoding the catalytic polypeptides (expansin proteins) from diverse plant or animal or microorganism genera that is available in order to compare the only disclosed genus in the instant case. Claims 14-16, 18 and 20 are rejected under 35 U.S.C. § 112 because no representative of species of polynucleotides are disclosed. Further,

not a single ‘oligonucleotide fragment’ or ‘primer’ of any size pertaining to SEQ ID NO: 1 is described.

Applicants have amended claims 14-16, 18 and 20 to describe the oligonucleotide fragment or primer of SEQ ID NO: 1 by structure, thus alleviating this rejection. Support for the amendment is found on page 19, lines 23-29. The methods of PCR and hybridization probes are well known in this art.

Claims 14-16, 18 and 20 were rejected under 35 U.S.C. § 112, first paragraph because the specification, while being enabling for a method for identifying nucleic acid sequence which encodes a protein with expansin activity, comprising the steps of isolating the nucleic acid sequence from a cDNA library by hybridization (under defined stringency conditions) using a DNA probe comprising all of the sequence of SEQ ID NO: 1, does not reasonably provide enablement for a method of identifying a nucleic acid comprising ‘an oligonucleotide of any size’...of SEQ ID NO: 1 (claim 14), or a fragment of undefined size for a ‘PCR primer’ or a ‘hybridization probe’ (claims 15-16), or obtaining an oligo-fragment(s) which encodes amino acid sequences of SEQ ID NO: 2-6 or their conservatively modified variants (claim 18) or designing a primer based upon the amino acid sequence of SEQ ID NO: 2. The Examiner states the factors most relevant to this rejection are the scope of the claims, unpredictability in the art, the amount of direction or guidance presented, and the amount of experimentation necessary.

Applicants have amended the claims to provide enablement for identifying a nucleic acid comprising an oligo-nucleotide by structure. Moreover, Applicants have deleted any recitation to conservatively modify variants. Additionally, Applicants have described the nucleotide primers by structure, thus alleviating this rejection. Support for the amendment is found on page 19, lines 23-29 and on page 33, lines 15-24. Methods such as determining an amino acid sequence from

the protein product, using this amino acid sequence to derive possible DNA sequences that would encode that amino acid sequence, and then using this DNA information to make probes to screen a library by, for example, hybridization, is well known to those of ordinary skill in this art.

Claims 14-16 and 20 were rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicant regards as the invention.

Claim 14, line 3, recites: "contiguous basis from SEQ ID NO:1". The Examiner states the spelling of 'basis' is incorrect.

Applicants have amended claim 14 by deleting the recitation "contiguous basis from SEQ ID NO: 1", thus alleviating this rejection.

Claim 14, line 5, recites "computer algorithm based assay". The Examiner states it is unclear what is meant by the phrase and the specification does not define the meaning of the phrase. Using the art accepted phrase or suitable modification will overcome this rejection.

Applicants have amended claim 14 by deleting the recitation "computer algorithm based assay", thus alleviating this rejection.

Claim 20, lines 3-4 recite "SEQUENCE ID NO: 2", which is not the accepted format. Rewriting the phrase to read as "SEQ ID NO: 2" is suggested to overcome this rejection.

Applicants have amended claim 20 by rewriting the phrase as suggested by the Examiner in acceptable format, thus overcoming this rejection.

Claims 15-16 are included in the rejection for failing to correct the defect present in the base claims.

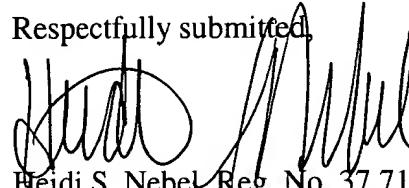
Applicants have amended claims 15-16 as well as the base claim which are now believed to be in form for allowance. Applicants respectfully request the Examiner for reconsideration and withdrawal of this rejection.

No fees or extensions of time are believed to be due in connection with this amendment; however, consider this a request for any extension inadvertently omitted, and charge any additional fees to Deposit Account No. 26-0084.

Attached hereto is a marked-up version of the changes made to the specification and claims by the current amendment. The attached page is captioned "Version with markings to show changes made."

Reconsideration and allowance is respectfully requested.

Respectfully submitted,

  
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**AMENDMENT — VERSION WITH MARKINGS  
TO SHOW CHANGES MADE**

**In the Specification**

Please amend the specification as follows:

On page 34 please replace the paragraph at lines 1 with the following:

An expansin, according to the present invention, is a purified protein generally having greater than about 60% sequence similarity and preferably greater than about 70% sequence similarity with the amino acid sequence disclosed in SEQ. ID. NO: 1. In a corresponding manner, the applicable DNA sequence [the] that expresses an expansin will have about 60% sequence similarity, and preferably greater than about 70% sequence similarity, with the nucleic acid sequence disclosed in SEQ. ID. NO: 1. The amino acid sequences identified in SEQ. ID. NO: 2 through SEQ. ID. NO: 6 are examples of sequences which meet the sequence similarity definition and thus are examples of expansins. Expansins equivalent to those described by this definition can be identified by their expansin characteristics, as explained throughout this specification, and all expansins are thus believed to be patentable equivalents.

On page 47 please replace the paragraph at lines 6, under Example 10 with the following:

[Effects of Cucumber Expansins on Cellulose Filter]

**Effects of Cucumber Expansins on Cellulose Filter**

Paper [Strips] strips of Whatman number 3 filter paper (Whatman Lab Sales, Hillsboro, OR) (2 mm by 10 mm) were cut and were clamped in the constant load extensometer as

described for cucumber hypocotyls sections. Extension was measured in 50 mM sodium acetate and in the same buffer containing protein fractions. Additionally, effect of expansins on the loss of mechanical strength of paper was measured by stress relaxation assay. For stress relaxation measurements, the paper was incubated in various pretreatment solutions and assayed while still wet, in the standard method.

On page 50 please replace the paragraph starting at line 25, under Example 15 with the following:

Western [Blott] Blot Analysis of Immunoreactivity between Expansin-like protein from Snail and Cucumber Expansin cEx-29.

Fig. 20 presents a Western blot of active HPLC-separated protein fractions from snail acetone powder, probed with antibody PA1 which was raised against cucumber [expansin- 29] expansin-29. The active fractions show a striking band at about 26kD, which is similar to (though slightly small than) cucumber expansin-29. This [results provide] result provides strong evidence that the wall extension activity found in the snail acetone powder is due to a protein with similar antigenic determinants as cucumber expansin-29.

On page 55 please replace the paragraph starting at line 28 with the following:

Isolation of a novel protein allows one to attempt the cloning of the gene coding for this protein using the standard approach of establishing an [amino acids] amino acid sequence of a fragment of the protein and designing oligonucleotides to screen the cDNA library. When cloned, the gene for one or more expansins will need to be expressed in a bacterial or other system to obtain sufficient quantities for the commercial usefulness of the ideas listed above. Cloning will also be a necessary first step for the commercial uses requiring genetic manipulation of the protein in transgenic plants.

On page 61 please replace the paragraph starting at line 2, under the heading "Abstract of the Invention" with the following:

[A new class of proteins and methods related thereto are presented. The proteins, which can be characterized as catalysts of the extension of plant cell walls and the weakening of the hydrogen bonds in pure cellulose, are referred to as expansin. Two proteins have been isolated by fractionation techniques from washed wall fragments of cucumber hypocotyls, referred to as "cucumber expansin-29" and "cucumber expansin-30" (abbreviated cEx-29 and cEx-30, with respect to their apparent relative masses as determined by SDS-PAGE). Moreover, three peptide fragments from the purified cEx-29 protein were sequenced, then oligonucleotide primers were designed to amplify a portion of the expansin cDNA using polymerase chain reaction with a cDNA template derived from cucumber seedlings, and then the PCR fragment was used to screen a cDNA library to identify full length clones. Another expansin protein has been isolated from oat coleoptiles (oat expansin oEx-29), while three additional expansin sequences have been identified in Arabidopsis and an additional two in rice. Expansins appear to be broadly distributed throughout the plant kingdom and can be identified in stem and leaf vegetables (i.e., broccoli, cabbage), fruit and seed vegetables (i.e., tomato), fiber crops and cereal (i.e., corn), and forest and ornamental crops (i.e., cotton). An expansin, generally, is a protein which has at least about 60% sequence similarity with the amino acid sequence shown in SEQ. ID. NO: 1, and preferably has at least about 70% sequence similarity with SEQ. ID. NO: 1. ]

A new class of proteins and methods related thereto are presented. This new class of proteins, called expansins, can be characterized as catalysts of the extension of plant cell walls and the weakening of the hydrogen bonds in pure cellulose.

In the Claims

14. (Amended)

A method of identifying a nucleotide sequence which encodes upon expression an expansin protein comprising:

obtaining [an oligonucleotide of contiguous basis from] a cDNA fragment having greater than about 70% sequence similarity to SEQ ID: 1;

using said [oligonucleotide] fragment to identify similar nucleotide sequences [through a] suspected to encode a protein with expansin activity by a hybridization[,] or PCR based assay[, or computer algorithm based assay of sequences suspected to encode a protein with expansin activity]; and thereafter

assaying the protein encoded by said identified sequence for expansin activity.

15. (Amended)

The[fragment] method of claim 14 wherein said fragment is a PCR primer.

16. (Amended)

The [fragment] method of claim 14 wherein said fragment is a hybridization probe.

18 (Amended)

A method of identifying a nucleotide sequence which encodes upon expression an expansin protein comprising:

obtaining [an oligonucleotide] a cDNA fragment [of contiguous bases] which [encode contiguous] encodes an amino [acids] acid sequence having greater than about 70% sequence similarity to

[from] SEQ ID NOS: 2-6 [or their conservatively modified variants]; using said fragment to identify similar nucleotide sequences through a hybridization or PCR based assay; and thereafter assaying the protein encoded by said sequence for expansin activity.

20. (Amended)

A method of identifying a nucleotide sequence which encodes upon expression an expansin protein comprising:

designing a primer to amplify expansin encoding DNA [based upon the amino SEQUENCE ID

NO:2] wherein said primer has greater than about 70% sequence similarity to SEQ ID  
NO: 2;

amplifying a cDNA fragment from said primer,

screening a cDNA library to identify a full length[,] coding sequence of an expansin protein.